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Assembly of Both the Head and Tail of Bacteriophage Mu Is Blocked in *Escherichia coli groEL* and *groES* Mutants

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Like several other *Escherichia coli* bacteriophages, transposable phage Mu does not develop normally in *groE* hosts (M. Pato, M. Banerjee, L. Desmet, and A. Toussaint, J. Bacteriol. 169:5504–5509, 1987). We show here that lysates obtained upon induction of *groE* Mu lysogens contain free inactive tails and empty heads. GroEL and GroES are thus essential for the correct assembly of both Mu heads and Mu tails. Evidence is presented that *groE* mutations inhibit processing of the phage head protein gpH as well as the formation of a 25S complex suspected to be an early Mu head assembly intermediate.

GroEL and its cofactor GroES belong to a subset of molecular chaperones called chaperonins. They control the folding of other polypeptide chains, protect them from aggregation, and regulate the assembly and disassembly of other protein complexes. *Escherichia coli groEL* and *groES* genes are essential at all temperatures (8). They form an operon which is constitutively expressed and is induced after heat shock. A 14-mer of the 57-kDa GroEL protomer associates with a 7-mer of the 10-kDa GroES protomer to form oligomers which act cooperatively in the folding of polypeptides. GroEL binds tightly to nonnative polypeptides. Upon association with GroES, by a mechanism involving ATP hydrolysis, GroEL discharges the polypeptide in a biologically active conformation (for reviews, see references 6, 17, 18, 29, and 42).

The first *groE* mutants were identified by their inability to grow bacteriophage λ or T4. Later, GroEL and GroES were demonstrated to also participate in the lytic cycle of many other bacteriophages. In all cases, the block caused by *groE* mutations is in morphogenesis. However, the steps affected differ from phage to phage. For λ and T4, the block is in head assembly (for reviews, see references 2 and 9), while for T5 and 186, tail assembly is the process requiring GroELS (21, 45).

Several head proteins, including gpB, are cleaved during λ head morphogenesis. The defective λ particles which accumulate in *groEL* or *groES* strains contain only unprocessed head proteins (19, 20, 22, 37). GroELS was shown to be necessary for the formation of the λ preconnector. This small 25S complex is the first detectable intermediate in λ head assembly (34). It consists of 12 subunits of λ protein gpB, is the precursor of the head-tail connector, and serves as the initiator for the assembly of the shell (26, 27, 35).

Like many other phages, Mu does not grow on some *groEL* and *groES* bacteria, although replication, transcription, and lysis occur normally in such hosts. This finding suggests that GroELS may also be involved in Mu morphogenesis (36). The assembly of Mu virions is under the control of 20 genes arranged in two clusters. The first contains the head genes *D*, *E*, *H*, *I*, *T*, and *J*; the second contains the tail genes *K*, *L*, *M*, *Y*, *N*, *P*, *Q*, *V*, *W*, *R*, *S*, and *U* (13, 14). gpT is the major coat protein. It forms the head shell (16, 38). gpD and gpE are suspected of being the Mu maturase components (5a). The protein encoded by gene *H* exists in two forms. One, gpH, has a molecular weight which corresponds to the size predicted from the nucleotide sequence of the *H* gene. It is found in a 25S complex which seems to be required for a very early step in head assembly. The second, gpH*, is found in heads and is derived from gpH by proteolytic cleavage of its C-terminal end. gpH processing occurs in assembled heads before DNA packaging (14).

We have analyzed Mu morphogenesis in *groEL* and *groES* hosts. Our results indicate that both head and tail assembly are affected. We have traced the main block in head morphogenesis to a defect in the assembly of the 25S complex and gpH processing.

MATERIALS AND METHODS

Media and general procedures. Bacteria were grown in LB and titrated on LA plates containing 1.2% Difco agar (33). Phage lysates were diluted in SM buffer (40) and titrated on lawns of sensitive bacteria (0.1 ml of an overnight culture in LB) poured with 2.5 ml of 0.7% LA agar on LA plates. The phages and bacterial strains used are listed in Table 1. The purified GroEL and GroES proteins were gifts from O. Fayet.

In vitro reconstitution. In vitro reconstitution experiments were performed as described by Giphart-Gassler et al. (13). The genotypes of the reconstituted phages were determined by marker rescue. Plaques of the phage whose genotype was to be tested were transferred with a toothpick to a mixed lawn of B178 and B178 lysogenic for one of the two amber mutants used in the in vitro reconstitution. The plates were incubated at 42°C. These tests showed a clear region of cell lysis if the reconstituted phage and the phage obtained by lysogen induction could recombine to yield wild-type phages.

Purification of phage particles. Phage particles obtained by thermal induction of lysogens were concentrated by polyethylene glycol precipitation and purified by ultracentrifugation through a CsCl gradient, followed by another ultracentrifugation through a sucrose gradient as described by Grimaud (14). Ultracentrifugation of total protein extracts was carried out as described previously (14).

Immunoblotting. Total protein extracts were prepared as described by Grimaud (14). Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (28). Immunoblotting was performed as described by Geuskens et al. (12) except that electrotransfer was carried out with a Bio-Rad apparatus for 4 h at 100 V for small gels and overnight at 50 V for large gels. Anti-gpH* antibody was used at a 1,000-fold dilution. Anti-GroEL IgG (Epicentre) was used at 0.2 μg/ml.

RESULTS

Head-tail assembly is blocked in *groEL* and *groES* hosts. Mu growth was tested on several *groEL* and *groES* hosts, among which some did and some did not allow the phage to form

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TABLE 1. Bacteriophages and bacterial strains used

Phage or strain	Relevant genotype	Reference or source
Phages		
Mu*cts*62pAp1		31
Mu*cts*62*Eam*1006		24
Mu*cts*62*Ham*1043		24
Mu*cts*62*Tam*1913		38
Mu*cts*62*Nam*1041		24
Mu*cts*62*Yam*1027		25
Mu*cts*62*Ham*7100		25
Mu*cts*62*Iam*4037		25
Bacteria		
B178	*galE* (Sup^0)	11
C600	*thi leu thr lacY tonA supE galK supE*	3
CG2241	B178*groEL44*	C. Georgopoulos
CG2243	B178*groEL140*	C. Georgopoulos
CG2244	B178*groES619*	C. Georgopoulos
KT14	B178*groES606*	O. Fayet

plaques (36a). Among the latter, two *groEL* strains (*groEL44* and *groEL140*) and one *groES* strain (*groES606*) (described in Table 2) were chosen for further investigation of the role of the GroELS chaperonin in Mu morphogenesis. These strains were lysogenized with Mu*cts*62pAp1 and induced at 42°C. Table 3 shows that under conditions where a wild-type lysogen produces lysates containing 2×10^9 phages/ml (data not shown), phage production from the *groE* lysogens was severely reduced, the number of plaque-forming phages varying from 2×10^5 to 6×10^6 phages/ml (i.e., 10^{-4} to 0.02 phage/bacterium).

In experiments where infectious particles were reconstituted in vitro by mixing a lysate produced by a head gene mutant (i.e., tail donor) with one produced by a tail gene mutant (i.e., head donor), Giphart-Gassler et al. (13) have shown that like other tailed bacteriophages, Mu assembles its heads and tails separately. These then join to form complete infectious virions. We used the same in vitro reconstitution assay to see which process or processes, i.e., head assembly, tail assembly, or both, is (are) deficient in *groE* strains. Mu*cts*62pAp1 lysates were prepared on each of several *groEL* and *groES* hosts (*groE* lysates). They were mixed with either a tail or a head donor lysate obtained by growing head mutant Mu*cts*62*Tam*1913 or tail mutant Mu*cts*62*Lam*1007 on a Sup^0 strain. The results, summarized in Table 3, show that when either heads or tails were added to a *groE* lysate, the number of plaque-forming phages always increased (by a factor of 2, to over 100). Always, however, such phages remained 10 to over 100 times less abundant than in control experiments where the head and tail donor lysates were mixed together. To test whether the *groE* lysates contain some inhibitory factor preventing the normal joining of fully functional heads and tails, we grew Mu*cts*62pAp1 on the *groEL140* host and mixed the resulting lysate with both head and tail donor lysates. The level of reconstitution was exactly the same as in the control experiment where no *groE* lysate was added (Table 3). The simplest interpretation of the reduced reconstituted phage yields obtained with *groE* lysates and heads or tails is that GroEL and GroES are required for both head and tail morphogenesis.

Reconstituted phages have the genotype of the head donor. We took advantage of this property to identify the true head donor in reconstitution experiments where a head donor lysate was mixed with *groE* lysates. Phages whose heads come from the *groE* lysate should be Am$^+$; those whose heads come from the head donor lysate should be amber. Table 3 shows that there were both amber and Am$^+$ particles among the reconstituted phages, the latter representing 9 to 76% of the reconstituted population. The presence of reconstituted amber phages indicated that the *groE* lysates contained some free active tails. The production of Am$^+$ phages suggested that *groE* lysates contained unstable or/and incomplete and hence noninfectious particles with a Mu*cts*62pAp1 genome that were rescued upon addition of a head donor lysate. Rescue could result from the addition of one or more factors present in the added lysate and coming from either the phage (e.g., proteins such as accessory proteins involved in the stabilization of the capsid, tail fiber proteins which are normally added after head-tail joining) or the bacterial host (e.g., GroEL and GroES). We tested the tail fiber hypothesis by looking for production of Am$^+$ particles after adding heads produced by a Mu*Sam* tail fiber mutant (15) to a *groE* lysate. Am$^+$ phages were as abundant as with other head or tail donor lysates (data not shown). This ruled out an involvement of tail fiber proteins. We also tested the possibility that Am$^+$ phage production resulted from the addition of GroELS present in the head donor lysate. Addition of purified GroEL and/or GroES to a *groE* lysate did not increase the formation of Am$^+$ phages (data not shown).

We next attempted to test head and tail morphogenesis separately in *groE* hosts. Three tail mutants (Mu*cts*62*Lam*1007, Mu*cts*62*Nam*1041, and Mu*cts*62*Yam*1027) and three head mutants (Mu*cts*62*Ham*7100, Mu*cts*62*Iam*4037, and Mu*cts*62*Tam*1913) were grown on the *groEL140* strain and on the *groES619* strain. Each resulting lysate was mixed with either a head donor (*Lam*1007) or a tail donor (*Tam*1913) lysate grown on a Sup^0 GroE$^+$ host. Table 4 shows that each head mutant grown on a *groE* host supplied tails as well as the control tail donor lysate did. In the reverse case, heads provided by a tail mutant grown on *groE* allowed for 2- to 20-fold less efficient reconstitution compared to the control head donor lysate.

TABLE 2. Characteristics of *groEL* and *groES* mutants

groE allele(s)	Codon	Sequence of mutations	Amino acid change	Thermosensitivity	Reference
L44	$\sigma^{70\text{-}35}$ region	TGGT**C**A--TGGT**T**A		Growth at 42°C severely impaired; does not grow at 43°C; restoration of growth by overexpression complete at 42°C and partial at 43°C	43
	Downstream *groES* stop	T**C**C--T**T**C			
	191	G**A**A--G**G**A	Glu→Gly		
L140	201	T**C**T--T**T**T	Ser→Phe	Grows at 42°C but not at 43°C; growth at 43°C restored by overexpression	43
S606 and *S619*	24	G**G**C--G**A**C	Gly→Asp	Growth at 42°C severely impaired; does not grow at 43°C; growth at 42 and 43°C restored by overexpression	30

TABLE 3. Reconstitution between *groE* lysates and tail or head donor lysates[a]

Allele	Titer on C600 (*sup*) (PFU/ml)	% Wild-type phages in reconstituted lysates	Control heads + tails (10^8)
EL140	3×10^6		
EL140 + heads	1.7×10^7	76	6
EL140 + tails	8×10^6	100	
EL44	6×10^6		
EL44 + heads	5×10^7	62	6
EL44 + tails	2.3×10^7	100	
ES606	1.6×10^5		
ES606 + heads	1.9×10^7	9	1.7
ES606 + tails	1×10^6	100	
EL140	1.6×10^5		
EL140 + heads + tails	8.1×10^7		1

[a] The efficiency of reconstitution of infectious phages from head donor and tail donor lysates was variable (10^8 to 8×10^8 phages/ml), probably due to the instability of head and tail donor lysates previously reported by Giphart-Gassler et al. (13). Variations in the titer of *groE* lysates alone could reflect the fact that cultures were not always induced at exactly the same optical density: to avoid any delay between culture lysis and use of the lysate in reconstitution assays, cultures had to lyse simultaneously and hence to be induced at the same time rather than at the same optical density. All experiments were repeated at least three times. The values shown here are those from the most representative experiments. *groE* lysates were obtained by growing Mu*cts*62pAp1 on B178*groEL140* (*EL140*), B178*groEL44* (*EL44*), or B178*groES606* (*ES606*). Head donor and tail donor lysates were obtained by growing Mu*cts*62*Lam*1007 and Mu*cts*62*Tam*1913, respectively, on B178. The proportion of Am^+ phages in reconstituted lysates was determined by stabbing isolated plaques on lawns of B178 (Sup^0) and C600 (*supE*). At least 50 plaques were tested in each experiment.

Thus active tails were produced in the *groE* host provided head assembly was blocked, but head assembly remained partially blocked in the absence of tail assembly.

***groE* lysogens produce free tails and empty heads.** To further characterize the defect in Mu morphogenesis, we purified, on CsCl and sucrose gradients, the particles present in Mu*cts*62pAp1 lysates obtained after thermal induction of *groEL* or *groES* lysogens (see Materials and Methods for the detailed protocol). Only particles with a 1.3-g/ml density, i.e., tails and/or empty heads (16), were detected. Sucrose gradient fractions were analyzed by SDS-PAGE, which enabled us to further distinguish defective heads (identified by the presence of gpT, the major head protein) from tail-related particles (identified by the presence of gpL, the major tail protein). All lysates contained head-related particles with a sedimentation coefficient of 100S, i.e., particles sedimenting like empty heads. Tail-related particles with a normal 90S sedimentation coefficient were also detected (Fig. 1A) (14).

The protein composition of the particles present in the head peak fractions of the sucrose gradients was investigated by SDS-PAGE (Fig. 1B). Empty heads and tails had very similar sedimentation coefficients and did not separate well. The head peak fractions displayed gpL and two tail proteins (average molecular size, 38 kDa) (Fig. 1B) (13) in addition to gpT. The protein composition of the tails present in *groE* lysates was thus no different from that of tails in wild-type lysates. gpT was the only head protein present in the *groE* lysates. Previous analysis (14) showed that complete Mu heads and most Mu empty heads contain both gpT and gpH*, a processed form of gpH. The empty heads produced in *groE* hosts showed no evidence of any gene *H* product (Fig. 1B). This was confirmed by im-

TABLE 4. Reconstitution of infectious phages from head or tail mutants grown in *groE* hosts[a]

Strain	Titer on C600 (*sup*) (PFU/ml)	Genotype of reconstituted particles	Control heads + tails (10^8)
Head mutants			
EL140Ham	$<10^2$		
EL140Ham + heads	1×10^8	*Lam*	5
EL140Ham + tails	$<10^2$		
EL140Iam	$<10^2$		
EL140Iam + heads	4×10^8	*Lam*	5
EL140Iam + tails	$<10^2$		
EL140Tam	$<10^2$		
EL140Tam + heads	2×10^8	*Lam*	1.6
EL140Tam + tails	$<10^2$		
ES619Ham	$<10^2$		
ES619Ham + heads	8.1×10^7	*Lam*	1
ES619Ham + tails	$<10^2$		
ES619Iam	$<10^2$		
ES619Iam + heads	8.4×10^8	*Lam*	8
ES619Iam + tails	$<10^2$		
ES619Tam	$<10^2$		
ES619Tam + heads	4×10^8	*Lam*	3
ES619Tam + tails	$<10^2$		
Tail mutants			
EL140Lam	$<10^2$		
EL140Lam + heads	$<10^2$		1.6
EL140Lam + tails	1.7×10^7	*Lam*	
EL140Yam	$<10^2$		
EL140Yam + heads	$<10^2$		3
EL140Yam + tails	5×10^7	*Yam*	
EL140Nam	$<10^2$		
EL140Nam + heads	$<10^2$		1.6
EL140Nam + tails	1.8×10^7	*Nam*	
ES619Lam	$<10^2$		
ES619Lam + heads	$<10^2$		1
ES619Lam + tails	4.7×10^7	*Lam*	
ES619Yam	$<10^2$		
ES619Yam + heads	$<10^2$		8
ES619Yam + tails	4.4×10^7	*Yam*	
ES619Nam	$<10^2$		
ES619Nam + heads	$<10^2$		8
ES619Nam + tails	7.1×10^7	*Nam*	

[a] The efficiency of reconstitution of infectious phages from head donor and tail donor lysates was variable (10^8 to 8×10^8 phages/ml), probably due to the instability of head and tail donor lysates previously reported by Giphart-Gassler et al. (13). Variations in the titer of *groE* lysates alone could reflect the fact that cultures were not always induced at exactly the same optical density: to avoid any delay between culture lysis and use of the lysate in reconstitution assays, cultures had to lyse simultaneously and hence to be induced at the same time rather than at the same optical density. All experiments were repeated at least three times. The values shown here are those from the most representative experiments. Head mutants used were Mu*cts*62*Iam*4037, Mu*cts*62*Tam*1913, and Mu*cts*62*Ham* 7100; tail mutants were Mu*cts*62*Lam*1007, Mu*cts*62*Yam*1027, and Mu*cts*62 *Nam*1041. Lysates of these phages were prepared by growing them on B178 *groEL140* and B178*groES619* (*groES619* is allelic with *groES606* [7a]). See Materials and Methods for determination of genotypes of the reconstituted phages. In all cases, titers on B178 (Su^0) were $<10^2$ PFU/ml.

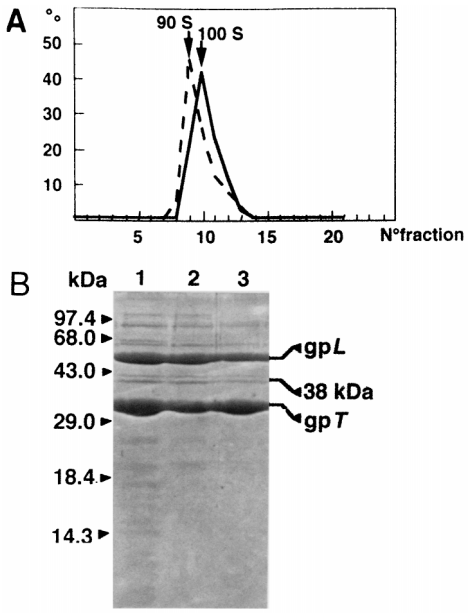

FIG. 1. Protein composition of Mu particles produced on *groE* hosts. (A) Sedimentation profiles of Mu particles produced by B178*groEL140*(Mu*cts*62 pAp1). Phage particles were isolated on CsCl gradients at a 1.3-g/ml density and run on a 10 to 50% (wt/wt) sucrose gradient at 45,000 rpm for 60 min at 5°C in a Beckman SW50.1 rotor. The sedimentation coefficients were estimated as described in reference 32. ——, relative percentage of gpT; – – –, relative percentage of gpL. The relative amounts of gpT and gpL in each fraction were determined by scanning Coomassie blue-stained SDS-polyacrylamide gels (see Materials and Methods for details). Results for B178 *groES606* (Mu*cts*62pAp1) and B178*groEL44*(Mu*cts*62pAp1) were the same as for B178*groEL140*(Mu*cts*62pAp1) and are therefore not shown. Sedimentation is from left to right. (B) Fractions containing the head peaks as identified in panel A were analyzed by SDS-PAGE (12.5% gel). The faint bands around 60 kDa were not present in all preparations and were thus probably contaminants. Lane 1, head peak fraction (no. 10) produced by B178*groEL44* (Mu *cts*62pAp1); lane 2, head peak fraction (no. 10) produced by B178*groEL140* (Mu*cts*62pAp1); lane 3, head peak fraction (no. 10) produced by B178*groES 606*(Mu*cts*62pAp1).

munoblotting analysis with anti-gpH* antibody of the same gradient fractions (data not shown).

To test whether the absence of gene *H* products in *groE* heads was due to a defect in gpH synthesis or gpH incorporation into the head, we probed total proteins obtained from induced *groE* lysogens with anti-gpH* antibody. gpH was detected in all extracts, whether derived from *groEL* or from *groES* strains lysogenic for Mu*cts*62pAp1 (Fig. 2). However, the processed form gpH* was always much less abundant in *groE* extracts. B178*groEL140*(Mu*cts*62pAp1) (Fig. 2, lane 3) and B178*groEL44*(Mu*cts*62pAp1) (Fig. 2, lane 2) displayed a small amount of gpH*, while in B178*groES606*(Mu*cts*62pAp1) (Fig. 2, lane 1), gpH* was not detectable. Synthesis of gpH was

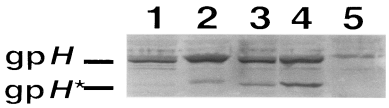

FIG. 2. Immunoblotting analysis of proteins synthesized in *groE* lysogens. Proteins were extracted from induced lysogens and separated on denaturing gels (12.5% acrylamide). The gels were probed with anti-gpH* as described in Materials and Methods. About 8 μg of protein was loaded in each lane. Lanes: 1, B178*groES606*(Mu*cts*62pAp1); 2, B178*groEL44*(Mu*cts*62pAp1); 3, B178*groEL140*(Mu*cts*62pAp1); 4, B178(Mu*cts*62pAp1); 5, B178.

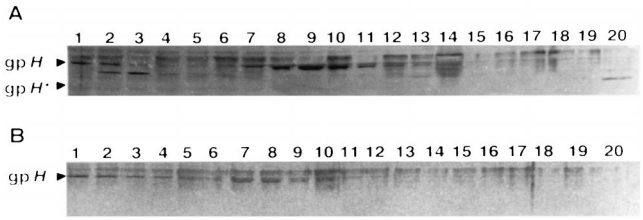

FIG. 3. Sedimentation analysis of particles present in induced *groE* lysogens. Total protein extracts prepared after induction of lysogens were run on a 10 to 40% (wt/wt) sucrose gradient at 50,000 rpm for 3 h at 5°C in a Beckman SW50.1 rotor. The gradients were fractionated from top to bottom, and the fractions were analyzed by immunoblotting with anti-gpH*. (A) B178(Mu*cts*62pAp1) extract; (B) B178*groEL140*(Mu*cts*62pAp1) extract. Sedimentation is from left to right. The cross-reacting band present in fractions 2 and 3 was previously observed in extracts prepared from nonlysogenic bacteria grown at 32°C and shifted to 42°C. The other cross-reacting band present in fraction 8 was identified as GroEL by immunoblotting with anti-GroEL antibodies (data not shown).

thus normal in *groE* hosts, but gpH processing and incorporation into the head did not proceed correctly.

The *groEL44* mutant has one mutation in the −35 region of the σ^{70} promoter of the *groELS* operon (Table 2). This mutation should play no major role in the phenotype of this mutant, as expression of the *groE* operon is mostly under the control of the σ^{32} promoter at temperatures between 30 and 43°C (44). In addition, *groEL44* thermoresistant revertants which retained the promoter mutation were isolated (43). To check that in our experiments expression of the *groELS* operon was not reduced by the promoter mutation, we used immunoblotting with anti-GroEL antibody to determine the amount of GroEL protein in our *groEL44*-derived strains. It was the same as that of GroEL in wild-type strains (data not shown).

***groE* mutations block an early step of Mu head assembly.** gpH was shown to be part of a 25S complex appearing as a likely very early intermediate in Mu head assembly (14). We looked for the presence of this complex in wild-type and *groE* strains lysogenic for Mu*cts*62pAp1. Crude extracts of the induced lysogens were loaded on sucrose gradients. After centrifugation, the gradients were fractionated and fractions were analyzed by immunoblotting with anti-gpH* antibody. In wild-type extracts (Fig. 3A), gpH migrated to the top of the gradient with unassembled materials (fraction 1) and to the position of the 25S complex (fractions 9 and 10). gpH* was found only at the bottom of the gradient (fraction 20). Fraction 20 was previously shown to contain also the Mu coat protein gpT. Most likely, only complete virions and head-related particles with a high sedimentation velocity migrate to that position (14). The situation in *groE* extracts was clearly different. gpH was present only in the top fractions (fraction 1) of the gradient (see Fig. 3B), indicating that in the *groE* strains, gpH incorporation into the 25S complex does not proceed normally. Very low amounts of gpH* could be seen in some *groE* extracts (Fig. 2), which led us to check the sucrose gradient fractions for the presence of the gpH truncated form. Although a low amount of gpH* sometimes appeared in the top fractions, despite several attempts, it could never be detected in the bottom of the gradients (data not shown). This finding suggested that in *groE* strains, gpH* was not associated with head-related structures and hence that gpH was not cleaved along the proper assembly pathway.

DISCUSSION

With Mu, both head and tail assembly are affected in *groE* hosts. Our in vitro reconstitution experiments (Tables 3 and 4)

show that only few active heads and tails are produced when Mu multiplies in *groEL* or *groES* hosts, suggesting that the GroELS chaperonin is required for the correct assembly of Mu heads and tails. This contrasts with observations on other phages where either head or tail assembly is affected (head assembly for λ, T4, and HK97; tail assembly for 186 and T5) (2, 5, 9, 21, 45).

λ and T4 mutants which have recovered the ability to form plaques on *groE* hosts can be readily isolated at frequencies ranging from 10^{-6} to 10^{-7} (10, 11). Yet, despite the use of various mutagens and several different *groEL* and *groES* alleles, we failed to find similar Mu mutants. This result is consistent with both Mu head and Mu tail assembly being blocked in *groE* hosts, as more than one mutation in Mu might then be required to overcome the *groE* defect, and double mutants might be too rare to be detected.

The major effect of *groE* mutation on Mu head assembly is at the level of the Mu head protein gpH. In Mu lysates prepared on *groE* hosts, we identified empty heads and inactive free tails. The defect in tails remains to be elucidated since they appeared unchanged in all of our analyses. The presence of empty heads suggests that head assembly is blocked before DNA packaging. Contrary to what happens in a $GroE^+$ host, the head protein gpH, although produced in a normal amount, does not assemble into a 25S complex, is not efficiently processed into its cleaved gpH* form, and is not incorporated into heads. This assembly defect is similar to that observed with Mu*Ham* mutants such as Mu*Ham*7100 which express no gpH and do not assemble the 25S complex (14, 16). Since this complex seems to be an early head assembly intermediate (14), blocking its formation would block gpH incorporation in the head and hence gpH processing which occurs only in assembled heads. *groE* mutations thus appear to cause a major and specific block in head assembly at the level(s) which involves gpH.

The role of GroELS in phage head assembly has been studied in great detail with phages HK97 and λ. The HK97 coat protein gp5 aggregates in *groE* hosts. A complex between GroEL and gp5 was isolated and used in vitro to show that GroELS promotes gp5 folding (5, 41). During λ head assembly, GroELS specifically interacts with gpB. Mutations bypassing the *groE* mutations have been found in gene *B*, and a biologically active GroEL-gpB complex has been identified (10, 11, 39). In these two cases, *groE* mutations cause a specific defect because the chaperonin is required to activate one particular head protein. The situation for Mu head appears similar since our results suggest that Mu gpH requires GroELS to assume its functional state. In *groE* hosts, because of delayed or incorrect folding, gpH would not be available for head assembly, and hence the resulting defect appears similar to that observed with mutant Mu phages which express no gpH.

It was proposed earlier that gpH could be a functional homolog of the λ gpB portal protein (14). The results cited above support that hypothesis and the similarities that exist between Mu and λ head assembly pathways.

How *groE* mutations can affect the production of native polypeptides and block Mu assembly. Mu lysates grown on *groE* hosts, although containing empty heads similar to those produced by Mu*Ham* mutants, also contain large amounts of defective free tails and smaller quantities of infectious phages, active free tails, and particles which become active upon combination with either head or tail donor lysates. In Mu*Ham* mutant lysates, in vitro reconstitution experiments did not provide any evidence for the existence of other types of phage-related particles besides empty heads (our unpublished results). The effect of *groE* mutations on Mu assembly thus cannot result from the sole absence of active gpH. The diverse defective particles produced, rather, reflect a requirement for GroELS at several morphogenetic steps or a deleterious effect of inactive gpH on several assembly steps. A direct effect of *groE* mutation on several morphogenetic steps implies that the chaperonin is required not only for the folding of gpH. This view is perfectly compatible with the properties of GroELS. Chaperonins play a general role in protein folding and seem to be required for the folding of many polypeptides. Horwich et al. (23) found that in bacteria lacking GroEL activity, 30% of the newly synthesized proteins aggregate. More recently, the flux of newly synthesized polypeptides through the chaperonin has been investigated. Under nonstress conditions, 10 to 15% of all newly synthesized polypeptides interact with GroEL (7). It is thus very likely that several Mu morphogenetic proteins require GroELS to reach their native state.

Among the different Mu-related particles produced in *groE* strains, the minor defective types likely derive from intermediates which escaped the major early defects in assembly of the 25S complex. These less abundant defective particles might also reflect a weaker dependence on GroELS of other phage proteins required for later assembly steps.

The *groE* mutants that we used in our experiments were characterized in great detail. Zeilstra-Ryalls and coworkers (43) showed that *groEL140* and *groEL44* strains which are thermosensitive for growth will grow at the nonpermissive temperature provided that the mutant proteins are overproduced. This finding suggests that the mutations, rather than knocking out the chaperonin's ability to fold the substrate protein correctly, decrease the folding rate. Biochemical analysis of the GroEL140 protein confirmed that it still binds substrate polypeptides normally but releases them abnormally slowly (1). Phage morphogenesis is known to require the assembly, in the right sequence, of a controlled amount of intermediates (4). If in *groE* strains the folding of some morphogenetic polypeptides is slowed down, the normal progress of the assembly steps will be disrupted. In phage λ, ε mutants, some of which carry a mutation in gene *E*, overcome the *groE* defect (11, 39). To account for this observation, it was proposed that in *groE* hosts, assembly of phage particles aborts because the slower release of the active form of one component disrupts the balance between this component and other morphogenetic proteins. By decreasing the rate of synthesis of gpE, the ε mutation would restore the balance between the slowly released component and gpE and hence restore assembly (11, 39). A similar process could account for the partial restoration of head assembly and total restoration of tail morphogenesis which we observed upon growing Mu tail or head mutants in a *groE* host. Very large quantities of late phage proteins are produced during the lytic cycle. In *groE* mutants, delayed release of GroEL-bound peptides could limit the amount of chaperonin available and hence the production of morphogenetic proteins competent for assembly. If production of either head or tail components is blocked (e.g., by an *am* mutation), more chaperonin would become available for those assembly steps which heavily rely on it.

Recently, the dependence on the chaperonin for folding of newly synthesized proteins has been investigated in *E. coli* (7). Three classes of proteins were distinguished: (i) a minor class consists of mostly small polypeptides which do not bind to GroEL; (ii) a second class includes the majority of the proteins which are largely independent of the chaperonin although about 5% of each of them bind to GroEL; and (iii) 10% of the newly synthesized polypeptides with sizes ranging between 25 and 55 kDa are strictly dependent on GroEL for their folding. In our study, the Mu gpH protein appears to strongly depend

on GroELS for function and hence would belong to the third class. Other Mu morphogenetic proteins seem to be affected by *groE* mutations to a lesser extent. These could belong to the second class, and only a small fraction of them would rely on GroEL for folding. In *groE* mutants, most gpH would be nonfunctional, leading to the accumulation of defective heads. Few functional assembly intermediates could be formed despite that primary block and proceed to further assembly steps which, depending on whether they do or do not rely on proteins belonging to the second class, will or will not proceed normally in the *groE* strain. This could lead to the formation of the minor types of defective particles that we observed. Our results are thus consistent with the view that the role of GroELS in Mu assembly mimics the general role of the chaperonin in the host bacterial cell.

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